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Synthesis of Hydroxyethylcellulose-g-Methoxypoly (Ethylene Glycol) Copolymer and Its Application for Protein Separation in CE

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ABSTRACT: Hydroxyethylcellulose-*g*-methoxypoly (ethylene glycol) (HEC-*g*-PEG) graft copolymers were synthesized through the etherification reaction between the hydroxyl group of hydroxyethylcellulose (HEC) and iodinated methoxypoly (ethylene glycol) (PEG-I), which was prepared on the basis of two-step reaction. Fourier transforms infrared spectrum (FTIR), nuclear magnetic resonance (NMR), thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC), and iodide oxidation method were used to prove the success of synthesis of graft copolymer. Furthermore, the comparative studies of electro-osmotic flow (EOF) and protein separation in bare-fused silica, HEC and HEC-*g*-PEG-coated capillary were performed in capillary electrophoresis (CE). The results showed that HEC-*g*-PEG-coated capillary presented efficient EOF suppression ability and excellent resisting protein adsorption ability.

KEYWORDS: cellulose and other wood products; coatings; copolymers; grafting; proteins

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INTRODUCTION

Recently some water-soluble cellulose derivatives have been found wide applications in areas concerned with oil recovery, medical, protective colloids, coatings, surfactants, thickeners, food additives, membranes.[1] The most valuable property of water-soluble cellulose derivatives is their solubility combined with chemical stability and nontoxicity to environment.

Hydroxyethylcellulose (HEC), as one of the water-soluble cellulose derivatives, is a nonionic fiber or powder solid carbohydrate polymer with white or lightly yellow color, stable chemical structure, and good biological compatibility.[2] It has good thickening, suspension, dispersity, emulsification and water protection properties.[3] Because of these excellent properties, it has many biotechnological, biophysical, and industrial applications,[4] such as oil exploitation, papermaking, coating, and polymerization area.[5]

Because in many cases the properties of HEC do not fit the needs for special applications, the grafting with other natural or synthetic polymers is a route to gain the desired properties. For instance, cellulosic graft copolymers can be used as antibacterial surfaces,[6] thermo-responsive smart materials,[7] membrane materials,[8] controlled drug delivery vehicles,[9] ion-exchange material, and sorption agents of heavy metals. Various conventional polymerization techniques for grafting polymer onto cellulose have been studied extensively.[10,11]

HEC exhibits the ability to adsorb onto a number of material surfaces through strong hydrogen bond interaction.[12] On the basis of the abundant reactive hydroxyl groups on HEC, HEC can be easily modified through its graft polymerization with hydrophilic vinyl monomers to derive new materials with improved properties.[13] Partly HEC grafting copolymers have been used as physical coating in fused-silica capillary inner wall for protein separation, such as HEC-*g*-PDMA (poly (*N, N*-dimethylacrylamide),[14] HEC-*g*-PDMAEMA (poly (2-(dimethylamino) ethyl methacrylate),[15] HEC-*g*-P4VP (poly (4-vinylpyridine)).[16] HEC can tightly adsorb onto the fused-silica capillary inner wall, however, resisting protein adsorption ability of HEC is not very well, comparing to another antifouling polymer PEG (poly (ethylene glycol)). Although excellent separation efficiency could be obtained by using PEG-coated capillary, PEG is ineffective to reduce the protein adsorption after several consecutive runs because PEG coating is easy to be washed away from the capillary inner wall by the buffer solution for its high hydrophilicity.

To improve the potential application of HEC in protein separation, we synthesized a novel HEC-*g*-PEG copolymer with good

antifouling property of PEG, and good adsorption ability of HEC through etherification reaction between hydroxyl of HEC and PEG-I.[17] Comparing to bare-fused silica and HEC-coated capillary, HEC-*g*-PEG-coated capillary have a better performance in protein separation efficiency and resolution.

EXPERIMENTAL

Materials

All water used in this experiment was deionized water and distilled three times prior to use. HEC (average $M_n = 9 \times 10^4$ g mol^{-1}, 80–125 mPa s, DS = 1.50, MS = 2.50) and PEG-OH (methoxypoly (ethylene glycol), average M_n = 350 g mol^{-1}, degree of polymerization = 8) were obtained from Aldrich. PEG-OH was dried by azeotropic distillation with toluene before using. Lysozyme from egg white (pI = 11.1, M_r = 14,300), cytochrome c from horse heart (pI = 10.2, M_r = 12,400), ribonuclease A from bovine pancreas (pI = 9.3, M_r = 13,700), α-chymotrypsinogen A from bovine pancreas (pI = 9.2, M_r = 25,656) were purchased from Sigma Chemicals (St. Louis, MO). Other reagents like tosyl chloride (TsCl), benzyl alcohol, disodium hydrogen phosphate, citric acid, etc., were all purchased from Sinopharm Chemical Reagent (Shanghai, China).

Synthesis of Methoxypoly (ethylene glycol) Tosylate (PEG-OTs)

PEG-OTs was prepared partly referred to the work of Yue and Cowie.[18] First, PEG-OH (0.056 mol) was added into a three-neck round-bottom flask containing anhydrous pyridine (10 mL), stirring in an ice bath under nitrogen protection, and then TsCl (0.102 mol) was added into the flask and the slurry was stirred for 5 h. Then CH_2Cl_2 (60 mL) was added into the flask and kept it at room temperature for 30 h with continual stirring. After that, the mixture was diluted with CH_2Cl_2 (60 mL), and the organic phase was subsequently washed with H_2O (2 × 100 mL), HCl aqueous solution (10 molL^{-1}, 100 mL), saturated $NaHCO_3$ solution (1.2 molL^{-1}, 100 mL) and then dried over magnesium sulfate. The solvent was removed by using vacuum distillation and a colorless liquid was obtained. Yield: 88%. The synthesis route is shown in Figure 1.

Synthesis of Methoxypoly (ethylene glycol) Iodide (PEG-I)

Sodium iodide (0.13 mol) was added into a three-neck round-bottom flask containing PEG-OTs (0.03 mol) in dry acetone (100 mL), and the mixture was refluxed for 30 h under nitrogen protection. The acetone was removed by using vacuum distillation. Then $CHCl_3$ (150 mL) and H_2O (150 mL) were added into the flask. The separated organic phase was washed subsequently with $Na_2S_2O_3$ solution (2.0 molL^{-1}, 150 mL), H_2O (2 × 150 mL), and saturated $NaHCO_3$ solution (1.2 molL^{-1}, 150 mL). Then the solution was dried over magnesium sulfate. The solvent was removed by using vacuum distillation and a slightly yellow liquid was obtained. Yield: 80%. The synthesis route is shown in Figure 1.

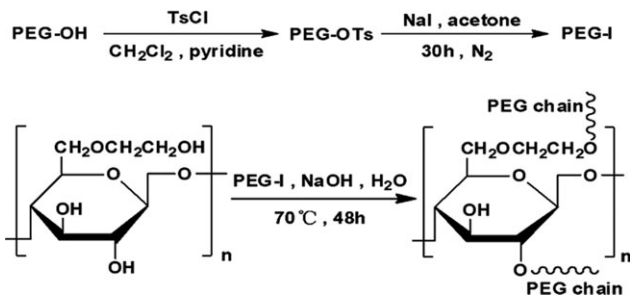

Figure 1. Synthesis procedure of HEC-*g*-PEG.

Synthesis of Hydroxyethylcellulose-g-Methoxypoly (ethylene glycol) (HEC-g-PEG)

HEC (0.1 g) was dissolved in NaOH aqueous solution (1.0 molmL^{-1}, 25 mL) in a one-necked round-bottom flask at room temperature with continual stirring. After 24 h, PEG-I (2.0 g) was added into the flask, and then the mixture was heated to 70°C and stirred for 48 h. Then the solution was precipitated in acetone to get rid of NaOH, residual PEG-I and new generated NaI (the precipitate is HEC-*g*-PEG). The acetone was removed by using vacuum distillation and then the HEC-*g*-PEG was dissolved into H_2O (25 mL) to make HEC-*g*-PEG solution. Then HEC-*g*-PEG solution was put into dialysis bag (cut-off M_n = 14,000 g mol^{-1}) and dialyzed for 5 days to get rid of slightly residual NaOH, PEG-I, and NaI. The HEC-*g*-PEG aqueous solution was freeze-dried for 2 days and then dried in a vacuum for 48 h at room temperature. The synthesis route is shown in Figure 1.

Fourier Transform Infrared Spectra (FTIR)

FTIR spectra of samples were recorded by EQUINOX55 (Bruker, Germany). Liquid PEG-OH, PEG-OTs, and PEG-I were directly coated on the crystal to prepare for FTIR experimental samples. Thirty-two scans were recorded in the range of 4000–500 cm^{-1} for each spectrum. Baseline was corrected for all spectra.

Nuclear Magnetic Resonance Spectroscopy (NMR)

^{1}H NMR spectra of samples were recorded by Bruker AVANCE-300 spectrometer (Bruker, Germany) at 40°C. Chemical shifts were referenced to deuterated chloroform ($CDCl_3$) and heavy water (D_2O).

Gel Permeation Chromatography (GPC)

M_n (number-average molecular mass), M_w (weight-average molecular mass), and PDI (polydispersity index) of HEC and HEC-*g*-PEG were determined by GPC measurements (LC-10AVP HPLC system, Shimadzu, Japan). The mobile phase was pure water with a flow rate of 0.50 mLmin^{-1} at 40°C, and poly(ethylene oxide) standard was used for calibration.

Thermal Gravimetric Analysis (TGA)

Thermal gravimetric analysis of PEG-OH, HEC, and HEC-*g*-PEG was carried out using a TA instrument Q5000 (TA, America) under nitrogen atmosphere. About 2.5 mg sample was heated from initial 40–450°C at a rate of 10°C min^{-1}. All samples were dried under vacuum at 40°C for 48 h prior to TGA measurement.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry analysis of PEG-OH, HEC, and HEC-*g*-PEG were studied by using DSC Q2000 thermal analyzer (TA, America) under a nitrogen atmosphere. Nearly 2.50

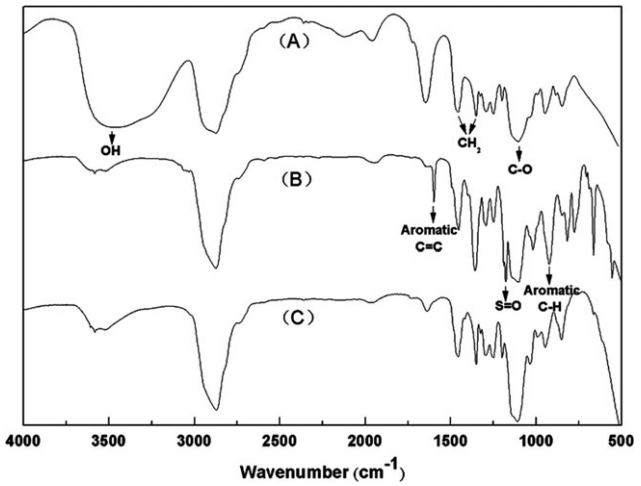

Figure 2. FTIR spectra of PEG-OH (A), PEG-OTs (B), PEG-I (C).

mg samples in an aluminum holder were heated from −80 to 150°C, then cooled to −80°C, and reheated to 150°C. Heating and cooling rate was 20°C min^{-1}. An empty aluminum pan was used as a reference material. All samples were dried under vacuum at 40°C for 48 h prior to DSC measurements.

Suppression of EOF in Capillary Electrophoresis

EOF measuring procedure was according to the protocol developed by Williams and Vigh.[19] A Beckman P/ACE MDQ system (Beckman Coulter Instruments) with a UV–vis detector working at 214 nm was used to determine the EOF (Electroosmotic Flow). Fused–silica capillaries (Yongnian Optic Fiber Plant, Hebei, China) with id/od of 75/365 μm and effective/total length of 30/40 cm were used for the estimation of EOF. All EOF measurements were carried out at 25°C with benzyl alcohol as neutral marker. To determine the ability of the copolymer to suppress the EOF, bare-fused silica capillaries, HEC-coated capillaries and HEC-*g*-PEG-coated capillaries were determined. HEC and HEC-*g*-PEG-coated capillaries were prepared as follows. First, the bare-fused silica capillary was preconditioned prior to its first use by flushing subsequently with 0.5 *M* NaOH for 5 min, H_2O for 5 min, 0.5 *M* HCl for 5 min and H_2O for another 5 min. Second, the capillary was flushed with HEC or HEC-*g*-PEG copolymer solution for 10 min and stood for 10 min to make capillary physical coating. HEC solution was prepared by dissolving HEC into H_2O to 0.2 $mgmL^{-1}$ (m/v) concentrations. HEC-*g*-PEG copolymer solution was prepared by dissolving HEC-*g*-PEG copolymer into H_2O to 0.2 $mgmL^{-1}$ (m/v) concentrations. EOF was measured in disodium hydrogen phosphate–citric acid buffers at pH 3.06, 4.12, 5.28, 6.16, 7.10, and 8.02.

Protein separation by Capillary Electrophoresis

The preparation of the coated capillaries for protein separation was the same as described above. Before injecting protein samples (basic protein: cytochrome *c*, lysozyme, ribonuclease A, α-chymostrypsinogen A, 0.4 $mgmL^{-1}$) for 3.0 s with 3447.5 Pa, the coated capillaries were rinsed with buffer at certain pH for 5 min to get pH balance. Four basic proteins separation was conducted in disodium hydrogen phosphate–citric acid buffers at pH 2.46, 3.06, 4.12, 5.28, 5.60, and 6.16. RSD (relative standard deviation) of migration time repeatability was determined by five continuous separations within 1 day.

RESULTS AND DISCUSSION

Synthesis of PEG-OTs, PEG-I, and HEC-g-PEG

Figure 2 shows FTIR spectra of PEG-OH (A), PEG-OTs (B), and PEG-I (C). —CH_2 (1456 cm^{-1}) and C—O (1107 cm^{-1}) stretching band can be seen in PEG-OH, PEG-OTs, and PEG-I. O—H stretching band (3522 cm^{-1}) can be seen in PEG-OH, but cannot be seen in PEG-OTs and PEG-I. Aromatic C=C stretching band (1598 cm^{-1}), S=O stretching band (1177 cm^{-1}), aromatic C—H deformation band (818 cm^{-1}) appear in spectra of PEG-OTs, but disappear in PEG-I spectra.

Figure 3 shows ^{1}H NMR spectra of PEG-OH (A), PEG-OTs (B), and PEG-I (C). As can be seen, δ = 2.42 ppm (s, 3 H, Ar-CH_3), δ = 3.40–3.80 ppm (m, 26 H, —CH_2—O—), δ = 7.30–7.80 ppm (2×*d*, 4 H, Ar-H) characteristic peaks appear in spectra of PEG-OTs. During the conversion from PEG-OTs to PEG-I, aromatic characteristic peak disappears, δ = 4.20–4.40 ppm (t, 2 H, CH_2-OTs) in PEG-OTs spectra transfers to δ = 3.20–3.30 ppm (t, 2 H, CH_2-I) under the influence of iodide. Both FTIR spectra and ^{1}H NMR spectra can prove that PEG-OH has been successfully transformed to PEG-I.

^{1}H NMR peak ownership of HEC and HEC-*g*-PEG is marked in Figure 4.[20–22] Figure 4(A) shows the ^{1}H NMR spectra of HEC and Figure 4(B) shows the ^{1}H NMR spectra of HEC-*g*-PEG. Comparing to HEC spectra, the chemical shift at δ = 3.22 ppm in Figure 4(B) is attributed to methoxy protons (a, —OCH_3) of PEG side chains, similar analysis method and characteristic peak change of cellulose acetate-*g*-methoxypoly (ethylene glycol) can be seen in the work of Yue and Cowie.[18] Additional evidence of PEG binding on HEC is obtained from oxidized reaction. Because of the iodide generation during the graft reaction process (HEC-OH + PEG-I→HEC-*g*-PEG + HI, HI + NaOH→ NaI + H_2O), after adding H_2O_2 solution into the reaction solution, the solution gradually changes to lightly yellow and this solution could make starch potassium iodide paper

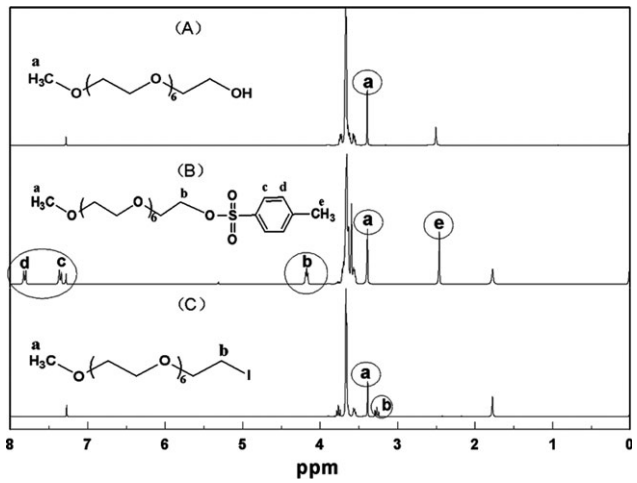

Figure 3. ^{1}H NMR spectra of PEG-OH (A), PEG-OTs (B), PEG-I (C).

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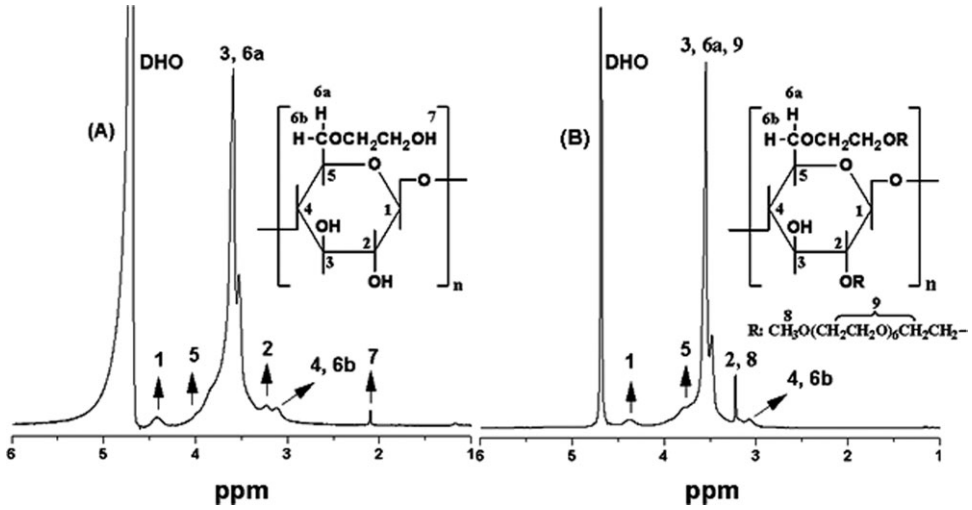

Figure 4. ^{1}H NMR spectra of HEC (A), HEC-*g*-PEG (B).

turn to blue because iodide is oxidized to iodine by H_2O_2 solution, which can prove that the solution contains iodide. So it can be concluded from above results that HEC-*g*-PEG copolymers have been synthesized successfully. Degree of substitution of hydroxyl groups in HEC to PEG is calculated according to the result of ^{1}H NMR integral (Figure 4). The integral sum of methyne (4, [tbond]CH) and methylene (6 b, $=CH_2$) in HEC [Figure 4(A)] are taken as standard (integral = 1.0), the integral sum of methyne (4, [tbond]CH) and methylene (6 b, $=CH_2$) in HEC-*g*-PEG [Figure 4(B)] are also taken as standard (integral = 1.0), then the integral difference between 3.10 and 3.30 ppm in HEC [Figure 4(A)] and HEC-*g*-PEG [Figure 4(B)] was calculated, which stands for the hydrogen content of methoxyl ($-OCH_3$) in HEC-*g*-PEG. After calculation, the degree of substitution is 0.24, which means that there is about 0.24 hydroxyl group was substituted in each glucose ring of HEC. The result of GPC measurements for HEC and HEC-*g*-PEG also showed that both M_n and M_w of HEC-*g*-PEG have increased obviously comparing to HEC.

Thermal Gravimetric Analysis (TGA)

As can be seen from Figure 5, PEG-OH decomposed in the range of 150–350°C and yielded a very small amount of residual mass (2.54%). PEG-NH_2 takes on the similar decomposition process.[23] HEC decomposed in the range of 200–450°C and yielded an amount of residual mass (15.35%), because of the formation of laevoglucose in the residual materials.[24] HEC-*g*-PEG decomposed in the range of 200–450°C and yielded an amount of residual mass (11.62%). The similar TGA analysis result of cellulosic graft copolymer can be seen in the work of Kadla et al.[25] HEC and HEC-*g*-PEG have nearly the same initial decomposition temperature. But the maximum decomposition temperature of HEC-*g*-PEG (372°C) has an ~12°C higher than original HEC (360°C), which is caused by the molecular weight increase of HEC-*g*-PEG than HEC. The shift of decomposition temperature is characteristic of the chemically modified cellulose.[26] It means that HEC-*g*-PEG has a better thermal stability than original HEC. The mass residue of HEC-*g*-PEG (11.62%) is lower than HEC (15.35%) due to the existing of PEG side chains, which has a very small residual mass (2.54%) during the decomposition process. Combustion heat of HEC-*g*-PEG (integral = 54.70) is higher than HEC (integral = 53.42), and this is caused by the PEG molecules (integral = 97.50) which can devote more entropy than HEC in the decomposition process. The result of TGA analysis proves that PEG has been grafted on HEC.

Differential Scanning Calorimetry (DSC) Analysis

Figure 6 shows the DSC curves of PEG-OH, HEC, and HEC-*g*-PEG. The common PEG-OH has a sharp endothermic peak at −9°C, corresponding to its glass transition temperature (T_g), with a fusion enthalpy of 70 J g^{-1} between −60 and 0°C. HEC shows a smooth curve during the given temperature scope. It proves that HEC has no thermodynamic change in this temperature scope. Similar DSC analysis result of HEC can be seen in Li et al.'s work.[27] For HEC-*g*-PEG copolymer, it shows an endothermic peak at 16°C with a fusion enthalpy of 15 J g^{-1} between 16 and 36°C, which is associated with the cleavage of PEG side chains from HEC. PEG decomposed temperature is shifted from −9 to 16°C under the influence of the covalent attachment of PEG on HEC.[28]

Suppression of EOF in Bare Fused–Silica, HEC, and HEC-g-PEG-Coated Capillary

EOF is a useful indicator in the clarification of the surface charges of the coating. Figure 7 shows EOF in bare-fused silica, HEC and HEC-*g*-PEG-coated capillaries at different pH, respectively. In the bare-fused silica capillary, the result shows that the EOF has a linear growth with the pH increases (EOF is close to zero at low pH, presenting a line increase between 3 and 6 and a sharp increase between pH 6 and 8), which is caused by the silanol group gradually protonating as the pH increases. In HEC and HEC-*g*-PEG-coated capillary, EOF is greatly suppressed depending on pH, especially at high pH. HEC and HEC-*g*-PEG can coat capillary inner wall by means of hydrogen bond interaction and shield the charge to control the EOF. At all the given pH, both of the coated capillary have a lower EOF than bare-fused silica capillary, and it takes on a relatively stable

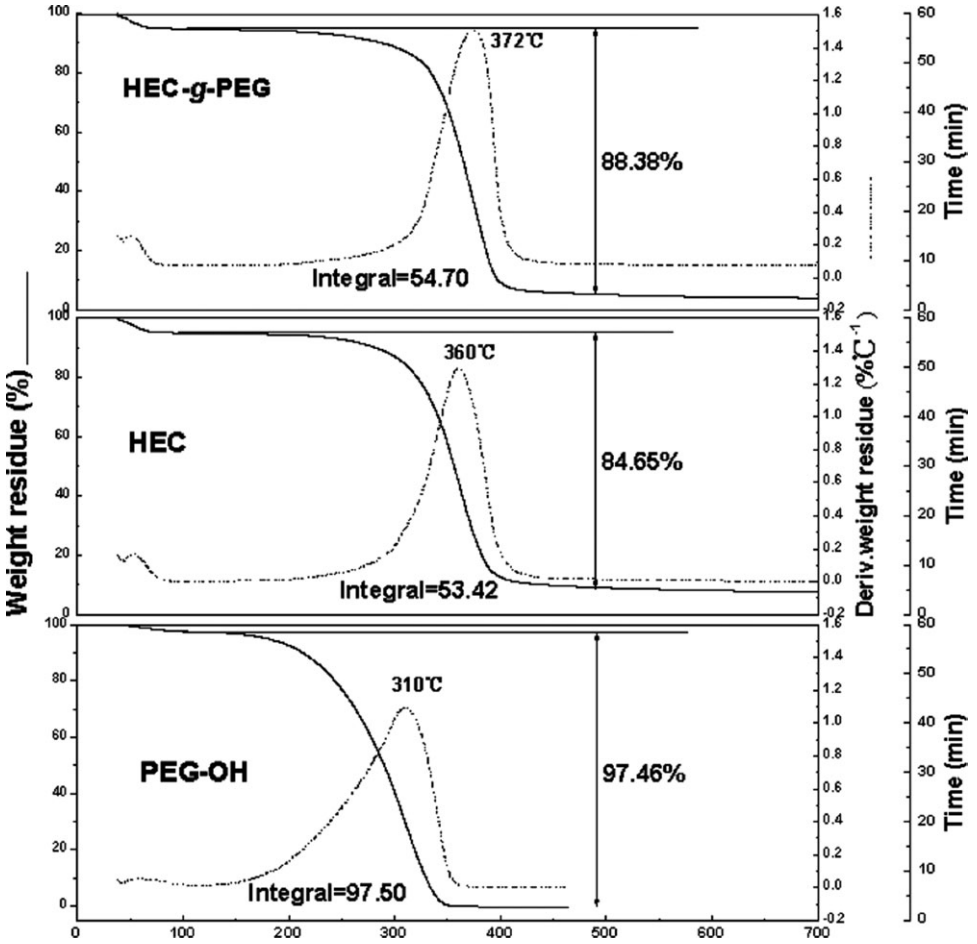

Figure 5. TGA curves of PEG-OH, HEC and HEC-*g*-PEG; programmed scan at 10°C min^{-1} under nitrogen atmosphere.

increase at high pH, not like the rapid increase of bare-fused silica capillary. Also at all pH, EOF of HEC and HEC-*g*-PEG-coated capillary is between 0 and 1.0 × 10^{-8} m^2 V^{-1} s^{-1}, these results are different from the HEC-*g*-PDMAEMA-coated capillary in our previous work.[15] For HEC-*g*-PDMAEMA-coated capillary,[15] it provides the anodic EOF at pH < 5.01 and cathodic EOF at pH > 6.14. As we know, PDMAEMA is a pH-sensitive polymer due to there are amine groups in the PDMAEMA chain, thus the degree of its protonation could be changed by changing the pH value of solution, and these result

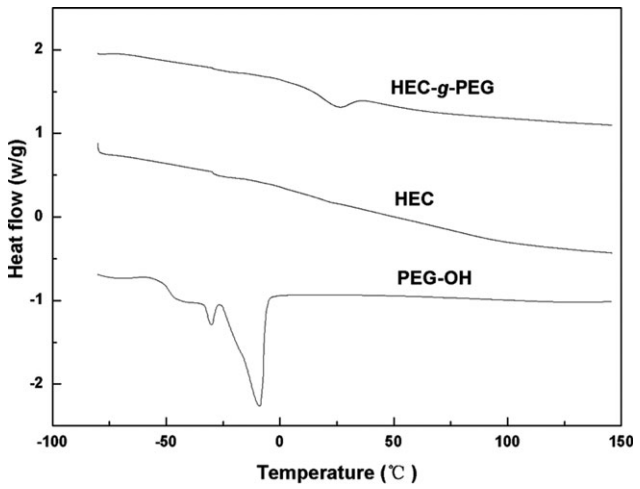

Figure 6. DSC curves of PEG-OH, HEC, and HEC-*g*-PEG; programmed scan at 20°C min^{-1} under nitrogen atmosphere.

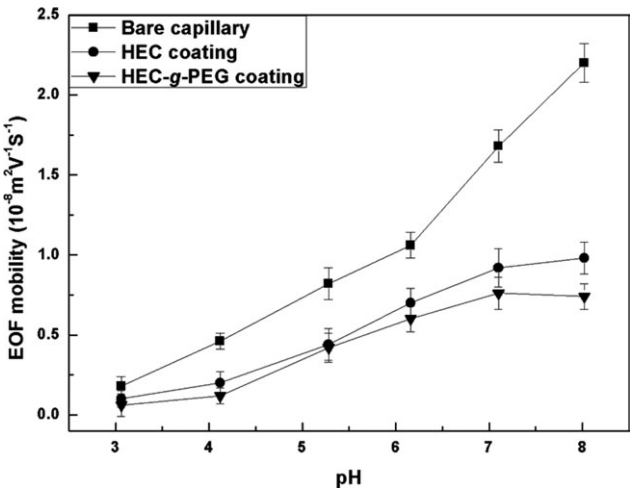

Figure 7. EOF as a function of pH. Comparison between a bare-fused silica capillary, HEC, and HEC-*g*-PEG-coated capillaries.

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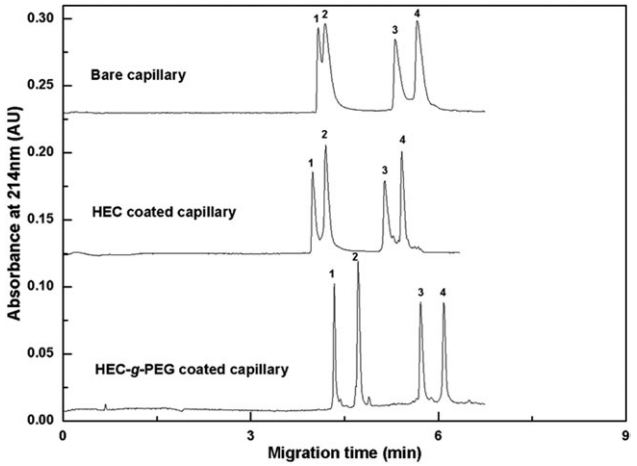

Figure 8. Separation of four basic proteins in bare-fused silica, HEC and HEC-*g*-PEG-coated capillary. Separation conditions: temperature, 25°C; separation voltage, +20 kV; detection, UV 214 nm; injection, 3447.5 Pa for 3.0 s; buffer, disodium hydrogen phosphate–citric acid of pH 3.06; sample: 0.4 mgmL^{-1} protein mixture. Peak identification: 1, cytochrome *c*; 2, lysozyme; 3, ribonuclease A; 4, α-chymostrypsinogen A.

in a reversed EOF generated. Whereas both of HEC and HEC-*g*-PEG belong to the neutral polymers and there are no charged groups in HEC and PEG part, thus there are no anodic EOF in HEC and HEC-*g*-PEG-coated capillary at the given pH scope, as shown in Figure 7. The result of EOF of HEC is accordant to the result of our previous work.[15] In suppressing EOF, HEC-*g*-PEG performs a little better than HEC. As we know both HEC and HEC-*g*-PEG copolymer can adsorb onto the capillary inner wall through hydrogen bond, however, for HEC-*g*-PEG copolymer, except for HEC adsorption onto the capillary inner wall, PEG also can adsorb onto the capillary inner wall via hydrogen bond between the ether oxygen of PEG and the Si—OH groups of the capillary inner wall.[29,30]

Protein Separation in Bare-Fused Silica, HEC and HEC-g-PEG-Coated Capillary

Generally, introducing a neutral coating between capillary and protein can resist protein adsorption. Meanwhile, the more the hydrophilicity of the neutral coating, the better the separation performance will be. The adsorption ability with capillary inner wall and the hydrophilicity of HEC-*g*-PEG can be increased simultaneously due to the existing of PEG chains comparing to HEC.[28] To prove that the new HEC-*g*-PEG coating has a better performance in separating proteins comparing to bare-fused silica and HEC-coated capillary, we make the contrast experiment in the same condition. Figure 8 shows four basic proteins separation results in bare-fused silica, HEC and HEC-*g*-PEG-coated capillary at pH 3.06, respectively. In bare-fused silica capillary, four basic proteins cannot be separated and the separation efficiencies are in the range of 43,000–99,000 plates m^{-1}. The protein peaks are low and have bad symmetry. In HEC-coated capillary, although the results are improved compared to the bare-fused silica capillary, four basic proteins also cannot be efficiently separated. Separation efficiencies of four basic proteins are in the range of 57,000–93,000 plates m^{-1}. For HEC-*g*-PEG-coated capillary, four basic proteins can be effectively separated with efficiencies in the range of 192,000–352,000 plates m^{-1} and the peaks have better symmetry. It shows that the separated efficiencies of HEC-*g*-PEG-coated capillary have a higher increase than bare-fused silica and HEC-coated capillary. The reason is that PEG side chains on HEC can not only resist protein adsorption on the capillary inner wall but also enhance the hydrophilicity of HEC as well. Therefore the theoretical plate number and the separation efficiency increase in HEC-*g*-PEG-coated capillary. From Figure 8, we can see that there is a longer migration time in HEC-*g*-PEG-coated capillary than in bare-fused silica capillary and HEC-coated capillary. As we know, during the process of protein separation in CE, there are two kinds of acting force which could promote this process. One is the electrophoretic force and another is EOF. At the given pH value, for bare-fused silica capillary, HEC-coated capillary and HEC-*g*-PEG-coated capillary, the direction of EOF is the same with the direction of electrophoretic force. Thus both electrophoretic force and EOF can make the positively charged proteins migrate from the anode to the cathode. Because HEC-*g*-PEG-coated capillary has a better suppression performance for EOF than bare-fused silica capillary and HEC-coated capillary at the given pH value, HEC-*g*-PEG-coated capillary has a lower EOF value comparing to bare-fused silica capillary and HEC-coated capillary, and the electrophoretic force is the same in these three kinds of capillary, so the migration time of proteins in HEC-*g*-PEG-coated capillary is longer than bare-fused silica capillary and HEC-coated capillary.

Table I. RSD[a] of Migration Time (n = 5) of Four Basic Proteins at Different pH in HEC-*g*-PEG-Coated Capillary

Protein	Cytochrome c		Lysozyme		Ribonuclease A		α-Chymotrypsinogen A	
pH value	*t* (min)	RSD (%)	*t* (min)	RSD (%)	*t* (min)	RSD (%)	*t* (min)	RSD (%)
pH 2.46	2.44	1.06	2.64	0.96	3.05	1.02	3.18	1.15
pH 3.06	3.46	0.97	3.73	0.92	4.44	1.04	4.67	1.08
pH 4.12	6.21	1.15	6.11	1.02	9.37	1.36	10.18	1.47
pH 5.28	6.74	1.12	6.36	1.13	10.62	1.28	11.38	1.33
pH 5.60	7.33	1.42	6.75	1.58	12.21	1.86	13.20	1.96
pH 6.16	7.91	2.02	7.20	1.94	14.11	2.05	15.64	2.45

[a]RSD $= \frac{1}{\bar{t}}\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(t_i-\bar{t})^2}$, $\bar{t}$ is the average value of migration time, t_i is migration time of every protein, and n is the times of protein separation.

Table II. Separation Efficiencies[a] ($n = 5$) of Four Basic Proteins at Different pH in HEC-*g*-PEG-Coated Capillary

Protein	Cytochrome c	Lysozyme	Ribonuclease A	α-Chymotrypsinogen A
pH value	N (plate m^{-1})	N (plate m^{-1})	N (plate m^{-1})	N (plate m^{-1})
pH 2.46	58,000	56,000	74,000	98,000
pH 3.06	361,000	200,000	230,000	360,000
pH 4.12	119,000	116,000	248,000	342,000
pH 5.28	152,000	146,000	133,000	115,000
pH 5.60	149,000	158,000	114,000	960,00
pH 6.16	113,000	134,000	81,000	56,000

[a] $N = 5.54 \times (\frac{\bar{t}}{w})^2$, $\bar{t}$ is the average value of migration time, w is peak width at half height, N is theoretical plate number.

Effect of pH on Separation

To investigate the applicability of HEC-*g*-PEG, separation of the four basic proteins (cytochrome *c*, lysozyme, ribonuclease A, and α-chymotrypsinogen A) are performed with pH range from 2.46 to 6.16. As shown in Table I, four basic proteins can be separated in 3.2 min at pH 2.46 and in 16.0 min at pH 6.16. It is noted that lysozyme has a longer migration time than cytochrome *c* at pH 2.46 and 3.06, whereas the migration order switches between cytochrome *c* and lysozyme at pH 4.12 and 5.28. In other words, increasing the pH from 3.06 to 5.28, there is a migration order switch between cytochrome *c* and lysozyme. Actually with the increase of buffer pH, the separation time increases correspondingly to the buffer pH, which indicates that the dissociation of the analytes is affected by buffer pH.[31] Otherwise, the change of buffer pH affects many aspects, including the surface charge of protein, the conformational properties of the protein, the dissociation of the silanol, etc. The change in migration order implies that pI can only be used as an indicator of the magnitude of net charge in the region close to pI.[32]

As shown in Tables I and II, for HEC-*g*-PEG-coated capillary, at pH 3.06, proteins are separated with separation efficiencies ranging from 361,000 to 200,000 plates m^{-1} and RSD are below 1.08; when at pH 6.16, proteins are separated with separation efficiencies ranging from 134,000 to 56,000 plates m^{-1} and RSD are below 2.45. RSD results show that HEC-*g*-PEG coating ($0.96 < RSD < 2.45$) had a better stability than HEC-*g*-PDMAEMA coating ($0.38 < RSD < 3.47$),[15] especially at higher pH value. This difference between HEC-*g*-PEG and HEC-*g*-PDMAEMA coating stability can be explained in the following way. When the pH increases, the number of negatively charged silanol groups (Si—O^-) on the capillary inner wall increase, and the protonation of the PDMAEMA also decreases (HEC-*g*-PDMAEMA coated capillary has an anodic EOF at $pH < 5.01$ and cathodic EOF at $pH > 6.14$), these lead to the decrease of static interaction between PDMAEMA and Si—O^-, and result in the decrease of the HEC-*g*-PDMAEMA coating stability.[15] HEC-*g*-PEG coating suffers less influence for the pH change comparing to HEC-*g*-PDMAEMA coating because HEC-*g*-PEG coating is a neutral coating, therefore it has better stability comparing to HEC-*g*-PDMAEMA coating as pH increases.

CONCLUSIONS

In this article, a well-defined HEC-*g*-PEG graft copolymer was synthesized successfully by etherification reaction. This copolymer showed a better thermal stability compared with HEC according to TGA and DSC analysis results. Because of the excellent adsorption ability of HEC and resistance protein adsorption ability of PEG, it was used as physical coating to suppress EOF and separate four basic proteins in capillary electrophoresis. This copolymer can tightly adsorb onto the inner wall of capillary by means of hydrogen bond interaction. In suppressing EOF, HEC-*g*-PEG coating performed well and kept EOF all below 1×10^{-8} m^2 V^{-1} s^{-1} at all given pH. In separating four basic proteins, it allowed the rapid and efficient separation within the pH range 2.46–6.16. It was noted that HEC-*g*-PEG-coated capillary obtained better EOF suppression performance, higher separation efficiency and better migration time repeatability than bare-fused silica and HEC-coated capillary.

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